Cross-Resistance to an Inhibitor of Chitin Synthesis, TH 60-40, in Insecticide-Resistant Strains of the House Fly

Eight insecticide-resistant strains of the house fly, representing organophosphorus, carbamate, and organochlorine resistance, were found to possess high levels of cross-resistance to the insect growth regulator (IGR) TH 60-40, an inhibitor of chitin synthesis. Levels of cross-resistance ranged from tenfold in a parathion-selected strain to considerably higher in a strain selected by *O*-ethyl *O*-(2,4-dichlorophenyl)phosphoramidothioate, indicating that IGR compounds are also subject to the risk of resistance development by target populations.

In the search for new, effective pest control agents, much attention has been focused on compounds which disrupt the normal processes of insect development. Most of the known synthetic insect growth regulators (IGRs) mimic the action of the *Cecropia* juvenile hormone (JH) (Slama, 1971) and have shown activity on many insect species (Jacobson *et al.*, 1972; Staal, 1972) including the house fly, *Musca domestica* L. (Jakob, 1973a,b; Cerf and Georghiou, 1972). A recently discovered class of IGRs, the benzoylphenylureas (van Daalen *et al.*, 1972), inhibits the synthesis of chitin, thereby causing abnormal endocuticular deposition and abortive moulting (Post and Vincent, 1973). Insecticidal activity has been reported against such pests as *Pieris brassicae* L., *Leptinotarsa decemlineata* L., various mosquito species, and *Musca domestica* L. (Wellinga *et al.*, 1973; Jakob, 1973b).

One major problem facing every potential insecticide is the possibility of development of resistance to it by the target pest. In this study, we report on the presence of cross-resistance to one of the more potent benzoylphenylureas, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea (PH 60-40; TH 60-40) (Mulder and Gijswijt, 1973), in certain insecticide-resistant strains of the house fly.

EXPERIMENTAL SECTION

The house fly strains utilized in this study included one susceptible (NAIDM-S), one organochlorine-resistant (DDT/lindane-R), one carbamate-resistant (OMS-15-R, selected by *m*-isopropylphenyl methylcarbamate), five organophosphorus-resistant [parathion-R; dimethoate-R; fenthion-R; Chlorthion-R (selected by *O,O*-dimethyl *O*-(3-chloro-4-nitrophenyl)phosphorothioate); and OMS-12-R (selected by *O*-ethyl *O*-(2,4-dichlorophenyl)phosphoramidothioate)], and a field strain (SK-R) colonized in 1971, also possessing high organophosphorus resistance (Georghiou *et al.*, 1972). With the exception of NAIDM-S and SK-R, all the strains have been under specific laboratory selection pressure for over 10 years and have attained maximal resistance to the selecting insecticide. Levels of resistance in these strains were reported elsewhere (Cerf and Georghiou, 1972). The flies were reared on standard CSMA media at 27° and 70% RH.

The compound was dissolved in tetrahydrofuran and applied to white prepupae (Fraenkel and Bhaskaran, 1973) in 0.5-μl volumes per insect. The treated prepupae were kept in screened paper cups under a 12:12 hr photoperiod at 27°. Humidity fluctuated from 60% during photophase to 95% during scotophase. There were 10 insects per replication and 10 replications per dose. Controls, treated with tetrahydrofuran, were maintained in every test and were utilized in correcting the experimental results. Activity was based on the number of flies successfully completing emergence from the puparium. Flies which were unable to free themselves from the puparium were also scored as affected.

RESULTS AND DISCUSSION

The visible response of the treated insects was failure to emerge from the puparium, ranging from minimal leg attachment to complete lack of emergence.

Table I. Susceptibility of Various Strains of the House Fly to TH 60-40 at Doses of 1 μg and 10 μg per Prepupa[a]

Strain	1 μg/prepupa		10 μg/prepupa	
	% mortal.	$S\bar{x}$	% mortal.	$S\bar{x}$
NAIDM-S	60	5.96[a]	87	4.73[a]
Parathion-R	32.3	6.39[b]	63.3	3.48[b]
Chlorthion-R	28.3	7.93[b]	58.6	5.37[b]
DDT/Lindane-R	21	5.18[b]	42	6.38[c]
Fenthion-R	20.9	6.01[b]	35.6	6.62[c]
OMS-15-R[b]	25	6.19[b]	32.4	5.28[cd]
Dimethoate-R	18.5	3.84[b]	32.3	6.18[cd]
SK-R	17.3	5.27[b]	28.1	4.12[cd]
OMS-12-R[c]	15.2	4.98[b]	18.5	4.95[d]

[a] Based on 100 insects per test. Any two means with the same roman superscript letter are not significantly different at the 5% level by Duncan's multiple range test. [b] *m*-Isopropylphenyl methylcarbamate. [c] *O*-Ethyl *O*-(2,4-dichlorophenyl)phosphoramidothioate.

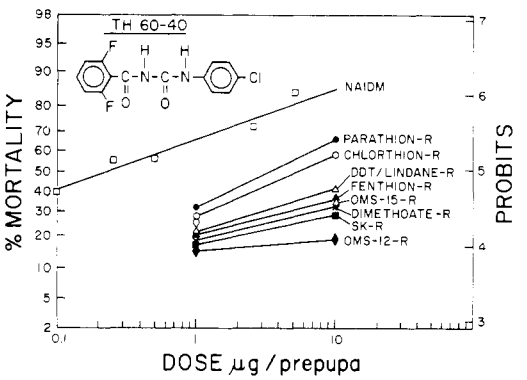

Figure 1. Comparative response to TH 60-40 in one susceptible (NAIDM) and eight insecticide-resistant strains of the house fly.

Base-line data for the NAIDM-S strain, presented in Figure 1, show a modest ED_{50} of 0.265 μg/prepupa and a relatively high ED_{95} of 70 μg/prepupa. The low slope of the regression line (b = 0.68) may indicate a slow rate of penetration through the cuticular layer.

Various levels of cross-resistance to TH 60-40 by the eight insecticide-resistant strains of the house fly were revealed by the application of the compound at two dosage levels, *e.g.* 1 and 10 μg/prepupa (Table I). The low dose provided ranking separation only between NAIDM-S and the resistant strains. But 10 μg/prepupa provided significant separation of the strains into three groups: the parathion-R and Chlorthion-R strains were the least resistant,

the DDT/lindane-R, fenthion-R, OMS-15-R, dimethoate-R, and SK-R strains were intermediate, while the OMS-12-R was the most resistant of all strains tested.

Previous reports have indicated that the benzoylphenylureas act only as stomach poisons (Mulder and Gijswijt, 1973; Wellinga *et al.*, 1973). However, by topically applying TH 60-40 in tetrahydrofuran to white prepupae, we have demonstrated that entry *via* the stomach route is not an essential requirement for toxicity.

The relatively high levels of cross-resistance toward TH 60-40, demonstrated by this study, may be disconcerting. Extrapolation of susceptibility data for the strain with the lowest resistance, parathion-R, reveals the presence of tolerance of approximately tenfold at the ED_{50}. Resistance in the OMS-12-R strain is obviously several fold greater. It is especially significant that the field strain, SK-R, is among the most resistant toward TH 60-40. Studies currently in progress are expected to elucidate the mechanisms of resistance to this compound. The detection of high levels of resistance in the house fly serves to emphasize the need for judicious use of new chemicals against presently susceptible populations, under conditions which minimize the degree of selection pressure.

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Received for review April 19, 1974. Accepted July 16, 1974. This work was supported in part by Grant R801837 from the Environmental Protection Agency, Washington, D. C.

A Specific Enzymatic Procedure for the Determination of Neurotoxic Components (Derivatives of L-α,β-Diaminopropionic Acid) in *Lathyrus sativus*

Lathyrus sativus seeds are used to adulterate some of the common dietary pulses such as *Cicer arietinum* (Bengal gram) and *Cajanus cajan* (Red gram) in India despite food laws. Chromatographic or other procedures available for detection of the neurotoxic or other components in *Lathyrus sativus* are nonspecific. In the present method, the neurotoxic components (derivatives of L-α,β-diaminopropionic acid) are separated by cation exchange chromatography and subjected to acid hydrolysis. The L-α,β-diaminopropionic acid formed was determined using the specific enzyme diaminopropionate-ammonia lyase. As little as 50 nm (5 μg) of L-α,β-diaminopropionic acid can be detected by the present method. The new procedure determines all acid-labile forms of bound L-α,β-diaminopropionic acid and extracts of *Cicer arietinum* and *Cajanus cajan* do not interfere in the procedure.

Lathyrus sativus (Kesari dhal) contains at least three neurotoxic components (Rao *et al.*, 1964; Rajamohan and Ramachandran, 1972; Rukmini, 1972) which may be partly responsible for the irreversible spastic paralysis among people whose diet contains substantial amounts of this legume (Ganapathy and Dwivedi, 1961; Nagarajan, 1969). Kesari dhal has been used as an adulterant despite food laws and a ban on interstate movement (Prevention of Food Adulteration Rules, 1955). Procedures to detect the presence of kesari dhal in Bengal gram flour (*Cicer arietinum*) or red gram (*Cajanus cajan*) have been reported in the literature (Nagarajan and Mohan, 1967; Dutta, 1965; Hartman *et al.*, 1973). The method based on detection of unique phenolic components present in *L. sativus* is nonspecific as other pulses such as *Vigna catang* (cow gram), *Lens culinaris* (Masur), and *Dolichos biflorus* (Horse gram) interfere. The chromatographic and electrophoretic method (Nagarajan and Mohan, 1967) based on detection of β-oxalyl-L-α,β-diaminopropionic acid (OXDAPRO), while being convenient and reasonably rapid, lacks the specificity of an enzymatic procedure and furthermore the ninhydrin reagent cannot detect α,β-dioxalyl-L-α,β-diaminopropionic acid (DIOXDAPRO). Five per cent adulteration of Bengal gram with *Lathyrus sativus* can be detected by the chromatographic method. The possibility that other ninhydrin positive components with the same chromatographic and electrophoretic mobility as β-oxalyl-L-α,β-diaminopropionic acid occur in natural material cannot be entirely ruled out although such an occurrence would be expected to be rare.

All the three neurotoxic components in Kesari dhal are derivatives of L-α,β-diaminopropionic acid (DAPRO) and are labile to acid hydrolysis yielding in the case of OXDAPRO and DIOXDAPRO nearly quantitative yields of DAPRO (Rao *et al.*, 1964; Rajamohan and Ramachandran, 1972). The "new toxic factor" (Rukmini, 1972) also contains DAPRO as an integral structural moiety but details about its complete chemical nature are not known as yet to predict its stability to acid hydrolysis. It was felt that a specific enzymatic procedure to determine DAPRO in acid hydrolysates of processed extracts of *Lathyrus sativus* would be helpful in determining the adulteration of other commonly used legumes with this pulse.

Furthermore, a combination of the paper chromato-